Almond Board of California
Annual Report - 2003-04

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ALMOND BOARD

Project Title: **Almond Variety Development**

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Objectives:

Develop improved pollinizers for Nonpareil, and ultimately, replacement varieties for Nonpareil and its pollenizers that possess self-fertility, improved disease and insect resistance and a range of bloom times and maturities. Goals for 2003-04 include:

1. Breed new selections through large-scale field crosses and seedling evaluations, characterize their cross-compatibility groupings using molecular as well as field tests. Continue to develop resistant cultivars for important almond diseases and Noninfectious Bud-failure.
2. Develop rapid selection techniques for self-compatibility, disease resistance (particularly Anthracnose and Alternaria), and NOW resistance.
3. Establish field trials of new self-compatible selections, particularly UCD36-52, a high quality Marcona type almond with high crack-out and good shell seal. Continue to monitor performance of 'Winters', low BF *Carmel* sources, and the *Nickels* hybrid rootstock presently in regional trials.

Summary

Breeding objectives for the Almond Variety Development Program include improved production consistency and efficiency. Improved production consistency is being pursued through the incorporation of self-compatibility in future almond varieties so that the crop is not so vulnerable to poor weather conditions during pollinations. Improved production efficiency involves the incorporation of disease and insect resistance to reduce grower inputs. Both objectives have required the identification of effective resistance/self-fertility genes and their transfer to California-type breeding lines. The recovery of promising genes for self-compatibility as well as resistance to naval orangeworm and diseases caused by *Coletotricum* (anthracnose), *Aspergillus* (aflatoxin) and *Salmonella* have been targeted. Concurrent with this germplasm development, rapid evaluation assays for greatly improving breeding efficiency have been developed for self-compatibility (with Dr. Dandekar), anthracnose (with Dr. Adeskaveg), and aflatoxin (with USDA scientists). Field crosses in 2003 reflect the transition from gene identification and

incorporation to the final development of the commercial almond varieties containing introduced genes combined with high horticultural quality. This focusing has allowed a greater number of crosses using fewer parents than have been needed in the past. Over 12,000 crosses were made in 2003 among 48 different crossing combinations (parents). Approximately 7000 seed have been recovered from these crosses of which over 5000 have been planted to the field. A total of over 24,000 seedling trees from controlled crosses are now being grown in breeding plots in Davis and Winters California. In addition to self-compatibility and disease/pest resistance, promising traits have been identified for tree architecture, bearing habit, productivity, and shell crack-out efficiency. Twenty advance selections demonstrating self-compatibility, improved nut quality, and/or improved insect and pest resistance have now been propagated to regional grower test sites in Calusa and Kern Counties.

Introduction

Commercial almond production is threatened by continuing losses of traditional insecticides, fungicides and insect pollinators. New domestic and international markets developed over several years of good production are lost when poor pollination conditions result in insufficient crop to meet established demand. Similarly, unique opportunities now exist to expand market demand by optimizing the phytonutrient benefits of almond in new California almond varieties while minimizing potential health and marketing risks such as aflatoxins, kernel cyanoglucosides and allergens. Thus, continued profitability of this industry relies upon dependable and quality production with reduced inputs. The recent releases to California growers of '*Winters*', a productive early 'Nonpareil' bloom pollinizer, and FPS#1, a low Bud-failure 'Carmel' clone covering the later 'Nonpareil' bloom, have achieved the initial breeding goals of effective pollinizers for 'Nonpareil'. The release of the 'Nickels' rootstock, along with continued identification/monitoring of low Bud-failure 'Nonpareil' clonal sources has made important early contributions to disease resistance. Current goals have now shifted towards breeding self-fruitful and disease (particularly Anthracnose, *Alternaria* and *Aspergillus* spp.) and Navel Orangeworm resistant varieties adapted to the Central Valley conditions and future marketing requirements. Breeding success depends upon locating sources of needed germplasm, the efficient indexing, testing and transfer of selected genes through controlled crosses, and the generation of large numbers of progeny from controlled crosses to ensure recovery of the rare, horticulturally superior individuals. New UCD varieties should have a durable paper shell with good seal to control worm, ant, fungal (*Aspergillus)* and bacterial (*Salmonella)* entry, while retaining high crack-out percentages. Varieties should also be free from Non-infectious Bud-Failure.

Establish regional trials.

Trees of the self-compatible selection ' UCD,36 - 52 ', which was selected as a substitute to the hard shelled Spanish variety *Marcona* (which is considered the premium almond in the European market), have been planted in grower trials in Calusa, Yolo, and Kern Counties, alongside Marcona standards. Besides being self-compatible, selection 'UCD 36-52' has the additional advantage of being a well-sealed almond but with crack-out ratios in excess of 55 percent (vs. approx. 35% for Marcona). Selection 'UCD 36-52' also shows resistance to NOW and greater field resistance to anthracnose disease than Marcona.

Field trials of the Winters almond, the Nickels rootstock, low BF Carmel sources, as well as previously planted advanced selections 2-19E, 25-75, 1-87, and others), located at both grower and regional variety trial sites, were further evaluated in 2003. The UCD Carmel clonal selection FPS #1 continues to show low bud failure expression in our long-term test plots in Kern County compared to traditional nursery sources which have shown consistently high BF expression (Fig. 1). The FPS#1 Carmel clone has been extensively used by the nursery industry since 2001. Some San Joaquin Valley Carmel orchards propagated from this source have recently shown levels of bud failure of 20 percent or greater, though these trees appear to be one to two vegetative generations removed from the FPS source and so erosion of the resistance to bud failure expression would be expected. The challenge to the California almond industry is to maintain low BF potential of the FPS clone even through vegetative propagations. Options include high-density hedgerows trees and the management, timing and handling of budwood. Regional studies are currently underway with cooperating

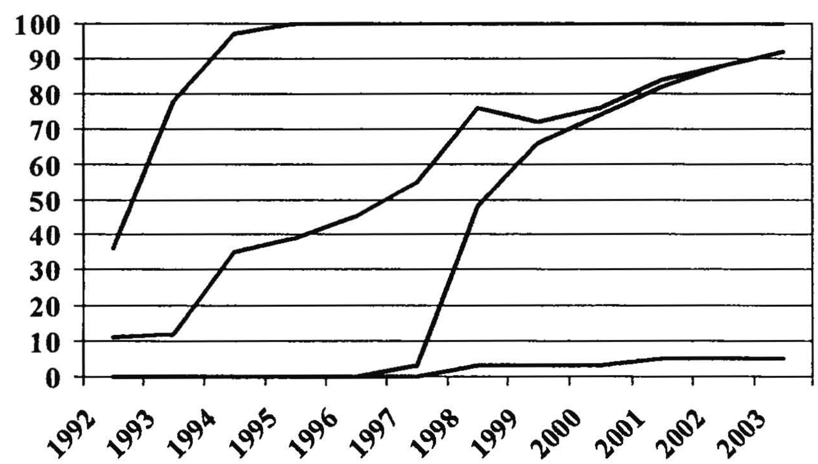


Figure 1. Incidence of buf-failure in FPS#1 source (Bottom right) compared to commercial sources over the past 10 years.

nurseries.

The Winters variety was developed as a pollinizer for the early Nonpareil bloom. Full-bloom in 2003 occurred approximately two days before Nonpareil full-bloom as compared to the Carmel variety which occurred approximately one day after Nonpareil. Good bloom overlap was consistent over the three regional variety trials at Butte, San Joaquin, and Kern counties, though some early straggle bloom was noticed on Winters in Kern County in 2003. Average bloom for 1996 though 2003 for Winters was 3.4 days before Nonpareil in Butte County as compared to 1.4 days after Nonpareil for Carmel (Fig. 2). When Carmel flowers after Nonpareil, the majority of pollen flows appears to move from Nonpareil to Carmel (i.e. Nonpareil performs as a better pollinizer for Carmel than vice versa).

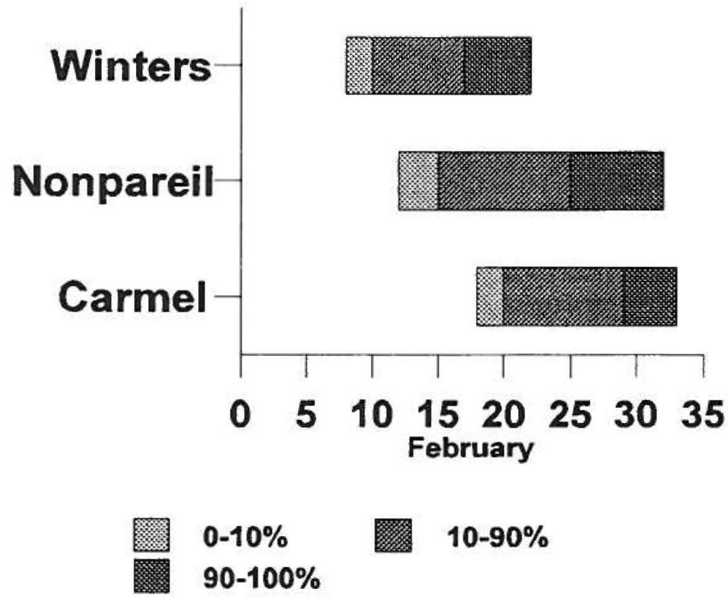


Figure 2. Comparison of bloom overlap of Winters and Carmel with Nonpareil at the Butte regional Variety trial (averaged over 5 years)

The Winters variety was the top yielder in Butte County in 2003 producing 3333 pounds per acre as compared with 2000 pounds for Nonpareil and 2330 pounds for Carmel. Performance in 2003 is consistent with average performance over the past five years were Winters has consistently outyielded both Nonpareil and Carmel (Fig. 3). The

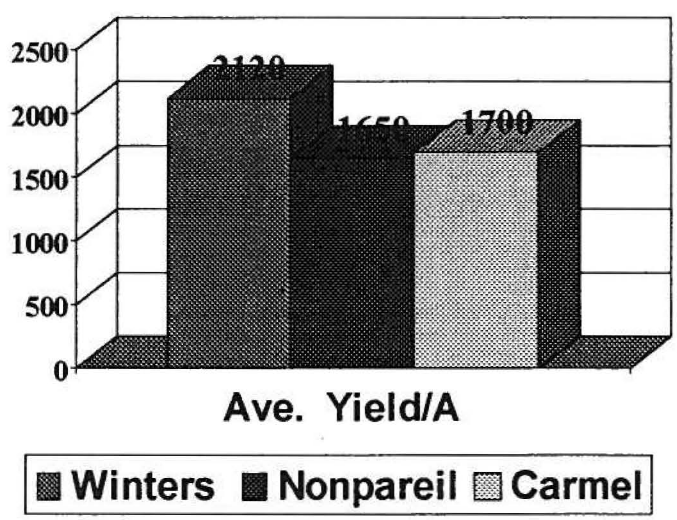


Figure 3. Yield comparison of Winters, Nonpareil and Carmel at the Butte Regional Variety Trial (average of 5 years).

Winters has consistently been the highest yielder in the Butte County regional variety trials since the first harvest in 1996 with the exception of 1998 yields where susceptibility to anthracnose resulted a severe yield losses (Fig. 4). The Winters variety is amongst the more susceptible to anthracnose disease, yet despite the presence of ample inoculum over the past six years in Butte County no serious outbreaks have occurred owing to identification and utilization of effective fungicides. Winters continued to show lackluster performance at the San Joaquin regional variety trial though this has been shown to be due to soil problems at the site (see 2002 report). San Joaquin yields in 2003 were proximally 1500 pounds per acre which was similar to the yields of the Nonpareil trees planted on either side of Winters and also suffering from the same soil problems. The yields of the Winters variety at the Kern County regional variety trials were also lackluster at 1927 pounds as compared to 2651 for Carmel and 2523 for Nonpareil. Much of the yield loss appears due to the susceptibility of the Winters variety to Alternaria leaf blight which is a serious problem at this site. Test plantings of the Winters variety have now been made at other Kern County sites were Alternaria is not a severe problem, to evaluate yield potential under more optimum conditions.

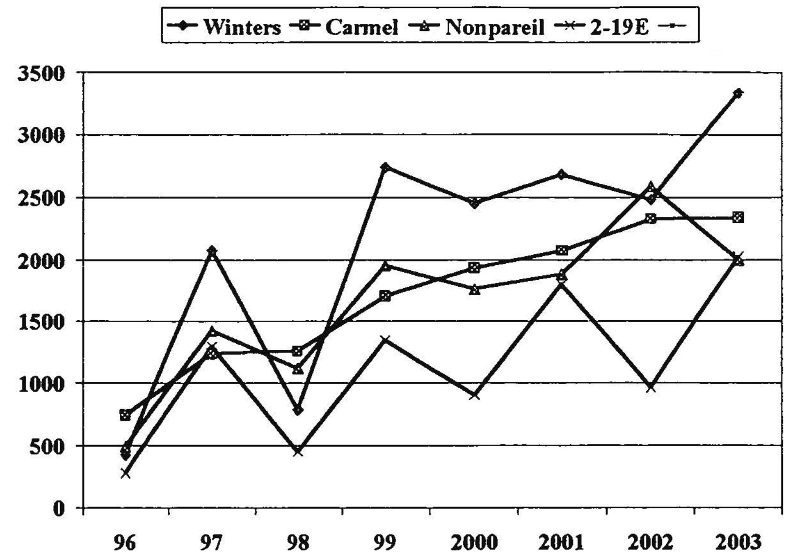


Figure 4. Yield performance of the Winters and comparison varieties at the Butte Regional variety Trials.

The highest yielding variety at the Kern County plots in 2003 was UCD selection UCD2-19E showing a remarkable yield of 4890 pounds per acre. The capacity of UCD2-19E to develop very high crops comes from its high spur density (being similar to the Price variety). As with the Price variety, UCD2-19E also shows a distinct tendency for alternate bearing (Fig. 5 and Fig. 4). Despite its alternate bearing tendency, however, UCD2-19E has shown one of the highest accumulated yields for this plot, producing 21,540 pounds over the last eight years as compared to 19,257 pounds per Nonpareil. Growers in this area have also had success in moderating the alternate bearing tendency through the judicial application of water and fertilizers during the 'on' years . Selection UCD2-19E shows good kernel and shell characteristics (Fig. 6) and its potential as a future cultivar release for California growers will depend on future progress in the cultural management of alternate-bearing varieties.

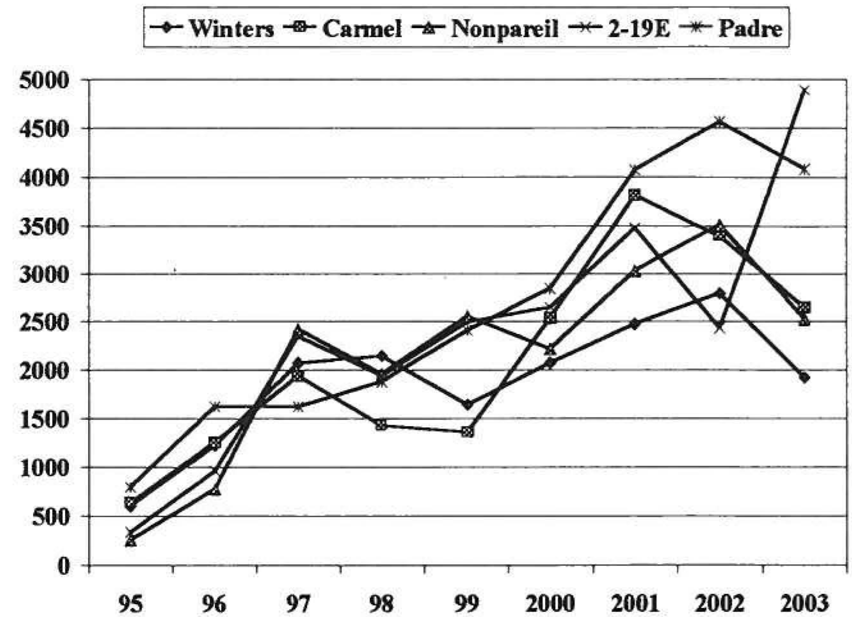


Figure 5. Yield performance of UCD Selection 2-19E and Winters variety compared to yield standards for the Kern Regional Variety trial.

A large-scale replicated field trial of 20 promising UCD advanced selections was also established in 2003-04 at the Billings Ranch in Kern County. Advanced selections included genotypes showing self-compatibility, improved pest resistance, improved kernel flavor, and/or improved kernel lipid quality (see summary in the last section of this report). A smaller nonreplicated trial of 12 advanced UCD selections was also planted at the Nickels Soils research plots in Calusa County.

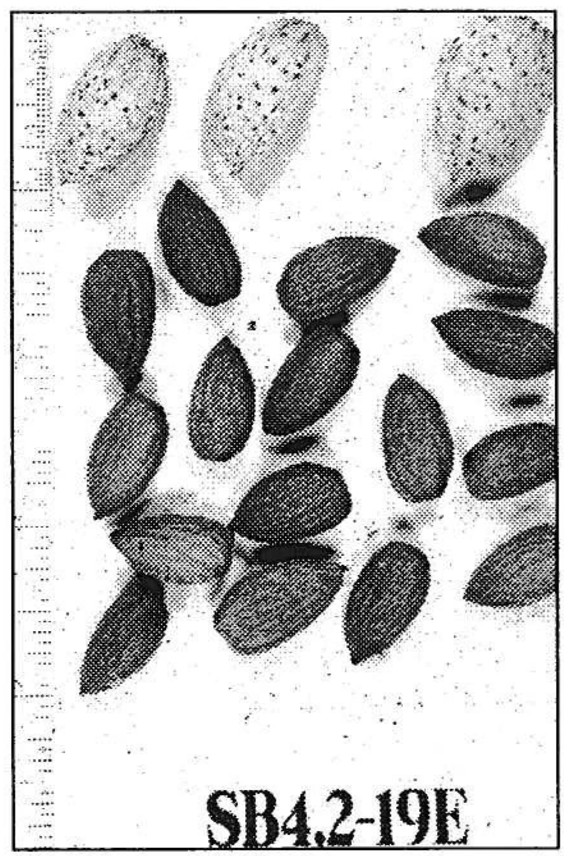


Figure 6. Kernel and shell characteristics of UCD 2-19E.

Develop rapid selection techniques.

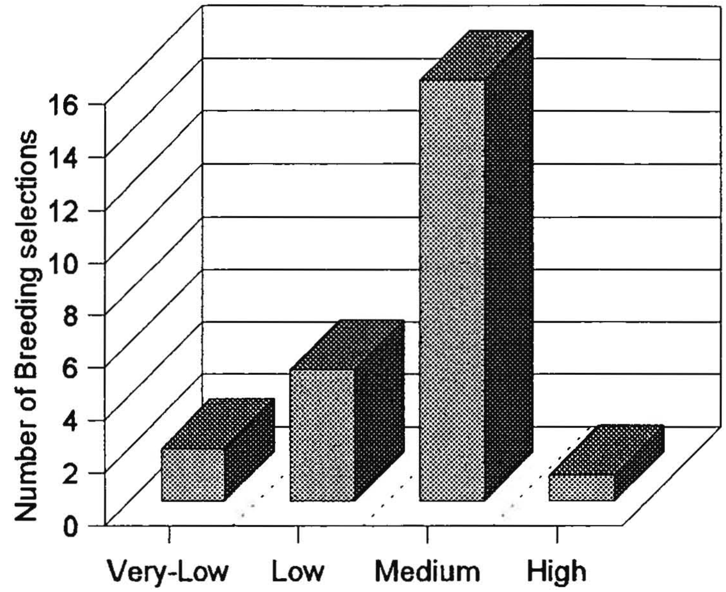


Figure 7. Distribution for susceptibility to anthracnose among 25 breeding lines tested.

Procedures for the rapid evaluation of susceptibility/resistance of targeted diseases are based on the inoculation techniques developed by Dr. Jim Adaskaveg and others. Anthracnose was evaluated following controlled inoculation of *Colletotricum* spores to intact developing almond hulls. The level of susceptibility was rated as very low to very high (Fig. 7) based on the extent of gumming/wounding on the hulls (Fig. 8) and/or the time to symptom development. Preliminary results have identified very low levels of susceptibility in UCD breeding lines, UCD 25-75 and UCD-97,2-2 40. The UCD 25-75 is one of our principal parent lines used for the transfer of high levels of self-compatibility, self-fruitfulness (high levels of self pollination without the need for honeybees) and resistance to aflatoxin producing Aspergillus. Selection UCD-97,2-2 40 is a more recent selection which has shown promise for transferring NOW resistance, high kernel crack out levels, early flowering, and high tree productivity to progeny from controlled crosses (see descriptions under NOW resistance).

Figure 8. Nut gumming as caused by anthracnose.

Similar inoculation and evaluation procedures are being tested for Alternaria, though disease resistance is mainly assessed through evaluation of natural infections in regional plantings of advanced selections and through inclusion in the *Disease Evaluation Block* at UC/Davis being managed by Dr. Jim Adeskaveg.

Figure 9. Almond kernel showing slightly shrunken cotyledon half which may be more susceptible to aflatoxin production and pellicle 'ink-staining'.

Aspergillus resistance/susceptibility was evaluated as the rate and extend of mold development on wounded mature kernels and, alternately as the level of toxin production following inoculation under optimal conditions (in collaboration with Dr. Noreen Mahoney, USDA/ARS Research Center, Albany, CA). Almonds tested include advanced breeding lines demonstrating good kernel quality and size as well as self-compatibility and/or promising levels of other disease resistance (such as previously discussed UCD 25-75). Several thousand aflatoxin field-infected kernels have been procured from cooperating handlers in 2003 in a study to characterize kernel characteristics which predispose almonds to aflatoxin. Traits being analyzed include seedcoat type and thickness, seedcoat integrity and chemical composition (particularly phenolic compounds), and seed development. Preliminary results suggest that when one of the pair of cotyledons in mature seed is less well developed (Fig. 9), that cotyledons is more susceptible to Aspergillus infection and aflatoxin production. If confirmed this relationship would be useful in characterizing the developmental/biochemical changes which predispose normally resistant almond tissue to toxin production. (Similar relationships have also been tentatively identified for preharvest ink-staining on the almond seedcoat).

Resistance/susceptibility to Navel Orangeworm has been evaluated in terms of the rate and extend of insect development following the placement of NOW eggs on hulls and nuts under controlled laboratory conditions. Greater future emphasis is being directed towards understanding the nature of almond shell fracture occurring at and following hull split. NMR imaging of the developing nuts of Mission (intact shell) and Nonpareil (shell fractures common) in combination with microscopic examination of tissue sections is clarifying the role of the vascular bundles leading to the abortive funiculus/ovule to subsequent shell fracturing. Components associated with NOW resistance also include hull, shell and kernel structure and biochemical composition. Our present understanding indicates that the most manageable and durable resistance to NOW and ant damage (as well as *Aspergillus* and *Salmonella* contamination) results from the development of an impenetrable shell structure. Breeding populations for testing the heritability of these resistances are now beginning to come into bearing and will allow a more precise estimate of genetic control. A promising selection from this project, UCD 97,2-240 confers a high level of resistance to NOW, ants, and Aspergillus causing aflatoxin, through a highly sealed shell (Fig. 10). The selection has now been planted in grower test plots and has been used as a recurrent parent in further controlled crosses. The high-crack-out, high shell integrity traits was originally transferred from the wild almond species *Prunus webbii* and appears highly heritable (i.e., the traits is readily transferred to progeny while allowing recombination with more desirable horticultural characteristics).

Figure 10. UCD Selection 97,2-240 demonstrating high shell integrity, high crack-out, and associated resistance to NOW, aflatoxin and Salmonella.

Rapid screening for self-compatibility is being pursued using the PCR techniques developed with Dr. Dandekar and colleagues which are specific to individual self-compatibility alleles (Fig. 11). The reliability of PCR based molecular markers for self compatibility have been verified through field crossing studies using controlled pollinations. The method has given consistent results for standard California self-incompatibility alleles though occasionally shows conflicting results for some of the self-compatibility/self-fruitfulness genotypes currently being incorporated into advanced breeding selections. Modified PCR technique techniques are presently being tested which may reduce this confusion while at the same time allowing more rapid marker detection.

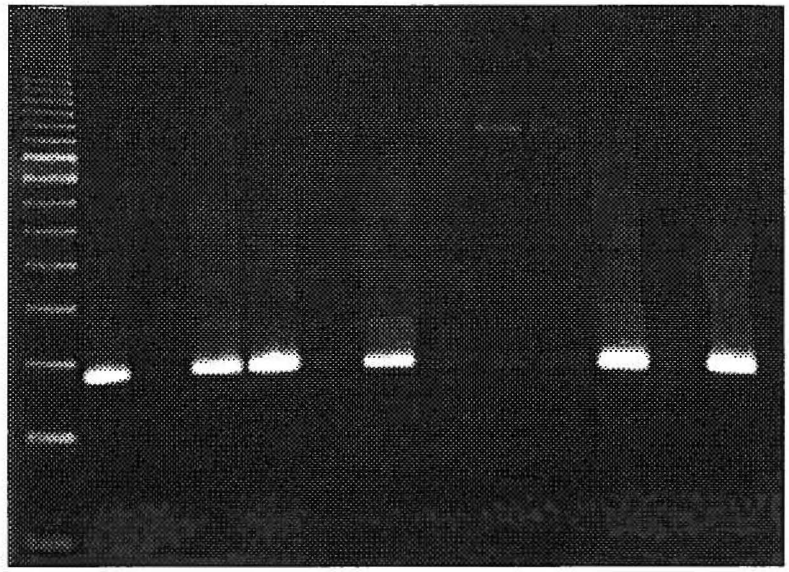


Figure 11. Rapid PCR based system for the accurate identification of cross-incompatibility grouping in almond.

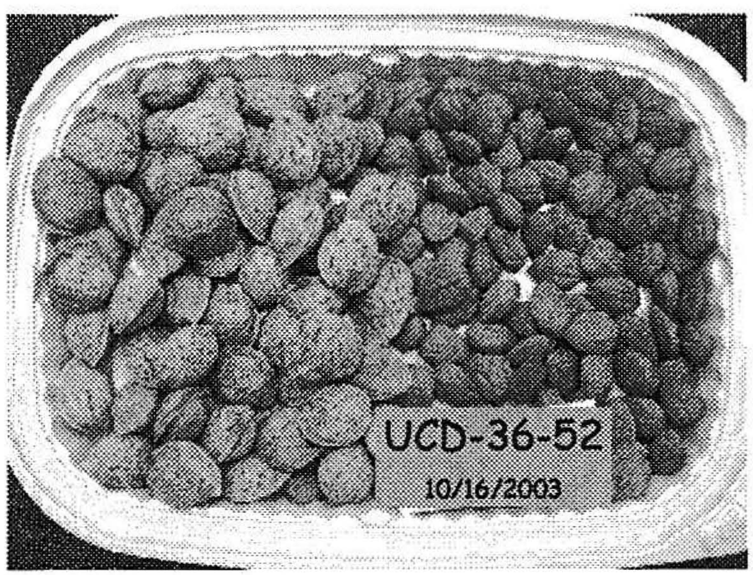
Breed new selections.

Within the past year, we have begun to recover advanced breeding lines sufficient in targeted traits to allow their use as parents for the next generation of breeding, emphasizing horticultural quality and yield. However, even with high quality and well-matched parents, the probability of a seedling progeny inheriting the best genes for the multitude of desired traits including self-compatibility, high nut quality, maximum yield potential, and disease/pest resistance is very low. To increase the likelihood of breeding such an elite individual, large populations of seedlings from controlled crosses need to be generated. In addition, since the best crossing combinations can only be verified by evaluating their progeny, a number of different crossing combinations need to be employed. Crossing goals for 2004-05 are 5,000 seedlings from approximately 14 different crossing combinations. Approximately 55% of controlled crosses in 2004 have been directed towards pest/disease resistance.

The bulk of field activities for 2003-04, involved the field evaluation of approximately 18,000 seedling trees developed from controlled crosses between parents with promising levels of disease resistance and or self compatibility. Goals include (1) the collection of tree and nut data to determine the value of various parental crossing combinations, and (2) the rouging-out or elimination of approximately 40 percent of seedling trees to allow a more detailed quality assessment of remaining crossing progeny in subsequent years.

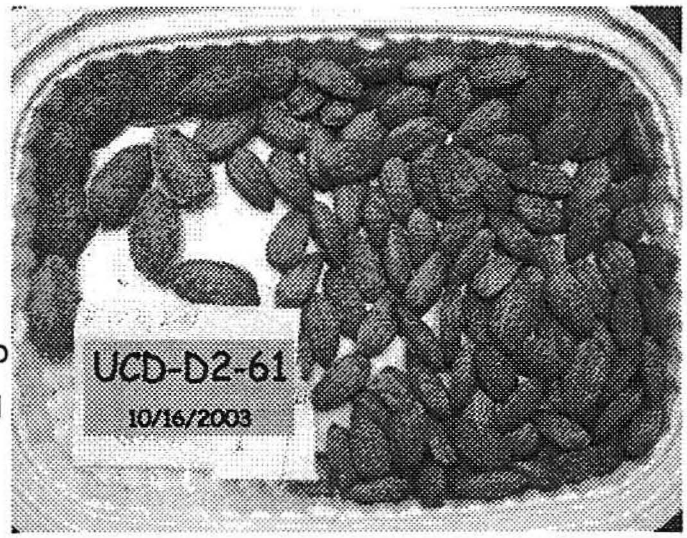
The most promising selections from the breeding program are being placed in regional grower plots to evaluate the performance under commercial conditions. As previously mentioned, in 2003-04 a large number of advanced selections were placed in regional plots: 20 UCD selections in Kern County, 12 UCD selections in Colusa County and two to four selections in Butte, San Joaquin, and Fresno Counties. Summaries of 6 of these advanced selections planted to both the Kern and Colusa plots are provided below.

UCD 36-52. The heart shaped, Marcona-like selection has the shape and high kernel quality of the premium Spanish variety Marcona. The kernels are also high in oleic acid content conferring a desirable buttery flavor, resistance to rancidity in storage, and phytonutrient benefits. The selection also possesses moderate to good levels of self-compatibility and resistance to NOW. Tree architecture and yield potential appeared good and flowering occurs shortly after Nonpareil. Susceptibility to anthracnose is comparable to



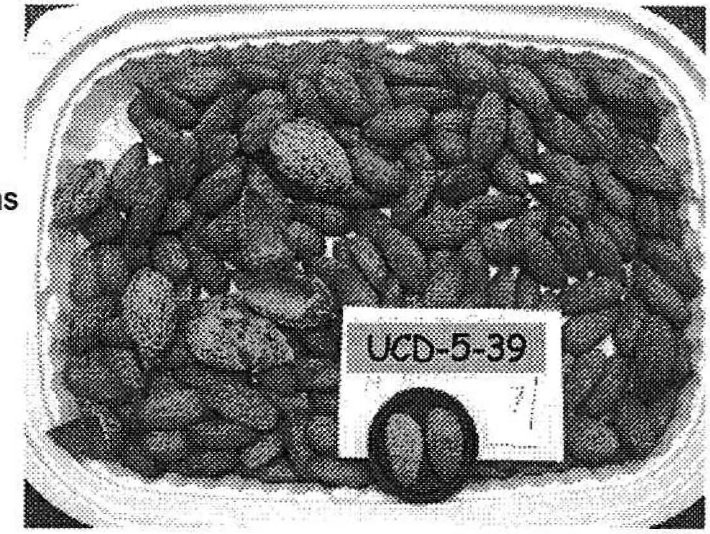
Nonpareil, being more resistant than the Marcona variety. The heart-shaped, high-quality nature of the kernel limit the marketing of this variety to specialty markets or to the well-established Marcona market in Europe. [The selection has been a perennial favorite of Almond Board evaluators since its superior flavor, heart shape and high proportion of monounsaturated fatty acids make it the poster-child (poster-kernel?) for almonds' excellent eating qualities and health benefits].

UCD D, 2-61. A very high-quality Nonpareil-type kernel and shell showing good yield potential. Bloom time is shortly after Nonpareil with harvest approximately three weeks later than Nonpareil. Parentage is from more traditional California breeding lines and so lacks both self-compatibility and improved disease resistance. Flavor has been rated to be comparable to superior to Nonpareil.



UCD 7-1 80. High-quality kernel with a slightly elongated shape somewhat between Nonpareil and Sonora. The selection shows moderate levels of self-compatibility as well as moderate resistance to anthracnose (preliminary results). The kernel shows a smooth, light-colored seed coat similar to the Carmel variety. Bloom time is approximately four days after Nonpareil while harvest is typically about ten days after Nonpareil. The self compatibility is derived from the wild peach-like selection and the tree still shows some peach-like characteristics which may ultimately compromise its yield potential.

<u>UCD 5-39.</u> Combines good quality Nonpareil-like kernel with good shell-seal. This selection is derived from more traditional California breeding lines and so lacking self-compatibility as well as improved levels of disease resistance. The bloomtime is with Nonpareil while harvest time is approximately two weeks after Nonpareil. The kernel texture and flavor have been rated as comparable to superior to Nonpareil and Carmel.



<u>UCD-F8, 8-4</u>. This selection combines high levels of self-compatibility, good tree structure and good yield potential. The self-compatibility was transferred from *Prunus mira*, a wild peach-like almond relative. Tree, foliage, and kernel characteristics show strong almond tendencies with no indication of the exotic lineage. This selection also shows improved resistance to NOW, anthracnose, and aflatoxin. The kernels are slightly smaller than Nonpareil and narrower. Bloom is approximately six days after Nonpareil with harvest approximately 16 days after Nonpareil.